关键词 羟基甾体脱氢酶类; 同工酶类; 棉酚; 低钾血症; 甾类

目的: 探讨棉酚诱发低钾血症机制. 方法: 从豚鼠肾脏皮质制备 11β-OHSD, 反相高效液相测定该酶活性. 结果: 依赖辅酶 I 的 11β-OHSD 的 V_{max} = 0.64 mmol·h^{-1}/g protein, K_m = 0.07 μmol; 依赖辅酶 II 的 11β-OHSD 的 V_{max} = 1.75 mmol·h^{-1}/g protein, K_m = 0.21 μmol. 棉酚对它们的抑制有显著差异, IC_{50}(95 %可信限) 前者为 50.2 (48.3 – 52.0) μmol, 后者为 1143 (1098 – 1188) μmol, 抑制常数 K_i 分别为 96 mmol·L^{-1}和 340 mmol·L^{-1}. 结论: 抑制依赖辅酶 I 的 11β-OHSD 是棉酚诱发低钾血症的更主要的生理因素.

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Effect of SR-140333, a neurokinin-1 receptor antagonist, on airway reactivity to methacholine in sedated rats[1]

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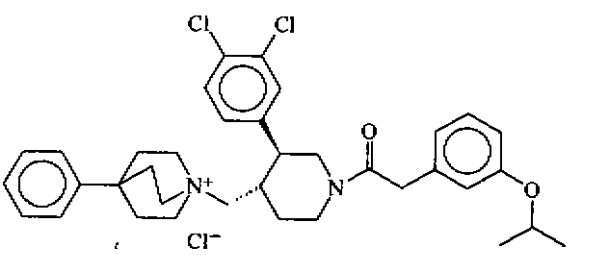
KEY WORDS SR-140333; methacholine chloride; atropine; neurokinin-1 receptors; albuterol; aminophylline; dexamethasone; trachea

AIM: To study the roles of neurokinins in the airway reactivity (AR) to methacholine chloride (MC). **METHODS**: The effects of (*S*)-1-(2-[3, 4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl)-4-phenyl-1-azoniabicyclo [2.2.2] octane·chloride (SR-140333), a neurokinin-1 receptor antagonist, on AR to inhaled MC in diazepam-sedated rats, and on MC-induced contraction of isolated tracheal spiral strips were observed. **RESULTS**: SR-140333 inhibited the increase in respiratory rate (RR) induced by MC aerosol (10 – 1000 μmol/m^3), and the ID_{50} for inhibiting the response to MC aerosol (1 mmol/m^3) was 4.9 μg·kg^{-1}(95 % confidence limits 1.4 – 17.2 μg·kg^{-1}). SR-140333 1 μmol·L^{-1} had no inhibitory effect on MC-induced tracheal contraction. Atropine blocked responses to MC both *in vivo* and *in vitro*. **CONCLUSION**: Endogenous neurokinins are involved in the AR to MC in rats, at least partly mediated via neurokinin-1 receptors.

Airway hyperreactivity to a wide variety of pharmacological and physical agents is one of the characteristics of asthma. Methacholine chloride (MC), is often used for measuring airway reactivity (AR) in humans[1] and animals[2]. Stimulation of sensory nerve C-fibers and the secondary release of tachykinins (TK), such as substance P (SP) and neurokinin A (NK-A), in the airways play an important role in AR to various stimuli[3], and antigen-induced airway inflammation[4,5] and bronchoconstriction[5,6]. MC or vagal nerve stimulation releases SP and NK-A from perfused guinea pig lungs[7,8]. Whether AR to MC relates to sensory nerve function and TK remains unclear. The present study was to clarify the contribution of endogenous TK to MC AR in rats using a neurokinin-1 receptor antagonist, SR-140333[9,10].



(*S*)-1-(2-[3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo [2.2.2]octane·chloride

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MATERIALS AND METHODS

Rats Sprague-Dawley rats of either sex weighing 154 ± *s* 23 g ($n = 226$) were from Laboratory Animal Center of Zhejiang Medical University (common, Certification No 22-9601018 conferred by Zhejiang Medical Laboratory Animal Administration Committee).

Drugs SR-140333 (Sanofi, France); diazepam (Tai-Xing Pharmaceutical Factory, Jiangsu); atropine sulfate (Ming-Sheng Pharmaceutical Factory, Hangzhou); salbutamol sulfate (Xing-Yi Pharmaceutical Factory, Shanghai); dexamethasone sodium phosphate (Xing-Chang Pharmaceutical Factory, Zhejiang); aminophylline (The 2nd Chang-Zhou Pharmaceutical Factory, Jiangsu); methacholine chloride (Sigma, USA). Methacholine was administrated in aerosol and the other 6 drugs were ip injected in various concentrations. All the drugs were prepared with freshly distilled water.

Measurement of AR to MC *in vivo* Having been sedated with diazepam 7.5 mg·kg^{-1} ip, the rat was placed in a plethysmograph (295 m^3) with air flow at 4 L · min^{-1}. Respiratory rate (RR) and plethysmograph pressure were monitored by a transverse piezoresistive pressure transducer (MPX 10DP, Motorola, USA). MC or saline aerosols was generated by an ultrasonic nebulizer (Model 402, Heli Medical Instrumental Factory, Shanghai) with an output of 367 μL · min^{-1}, and passed into the rat chamber where the aerosol concentration was 106.6 mL/m^3.

RR was taken as an index for AR to MC because it was a stable and sensitive parameter in our preliminary studies in rats (unpublished data) and guinea pigs[11]. Rats were challenged with MC aerosols 10, 100, 1000 μmol/m^3 for 2 min at intervals of 30 min. The increases in RR (ΔRR) within 5 min were recorded and the concentration of MC causing 30 % increase of RR (PC_{30}) was calculated [12].

Rats were ip injected with SR-140333 1.0, salbutamol 1.0, aminophylline 10, dexamethasone 0.5, atropine 1.0 mg·kg^{-1}, or saline 1.0 mL · kg^{-1} and 30 min later AR to MC was measured. In 31 rats (divided into 3 groups), SR-140333 (1, 3, 10, 30, 100 μg·kg^{-1}), atropine (3, 10, 30, 100 μg·kg^{-1}) and salbutamol (1, 3, 10, 30 mg·kg^{-1}) were ip injected, and 30 min later ΔRR was determined after inhalation of MC aerosol (1 mmol/m^3, for 2 min). The dose of the 3 drugs required to inhibit 50 % of the maximal ΔRR (ID_{50}) was calculated[13].

Tracheal contractile response to MC *in vitro* The tracheal spiral strips of rats were equilibrated in oxygenized Krebs' solution at 37 ℃ with a resting tone of 1 g for 1 h. With or without the pretreatment of atropine (0.1, 1.0 nmol·L^{-1}) or SR-140333 (0.1, 1 μmol · L^{-1}), gradually increasing concentrations of MC (0.01 – 100 μmol · L^{-1}) were added. The contractions were measured with a force displacement transducer (SMU-A, Department of Pharmacology, Shanghai Medical University). The maximal contraction (E_{max}) and the concentration of MC ($[MC]_{50}$) that produce 50 % of the maximal contractile effect were calculated ($pD_2 = -\lg[MC]_{50}$).

Statistical analysis The *t* test was done on a computer software (SPSS 6.0 for Windows, 1993, SPSS Inc, USA).

RESULTS

The drugs used did not alter the baseline RR. SR-140333, salbutamol, aminophylline and atropine markedly decreased maximal ΔRR after MC inhalation and the PC_{30} of MC was elevated, but dexamethasone did not change both parameters (Tab 1).

Tab 1. Effects of 5 drugs on the responses to MC in sedated rats. $x \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

Drugs/ μg·kg^{-1}		*n*	Baseline RR (breaths ·min^{-1})	Maximal ΔRR (breaths ·min^{-1})	– lg PC_{30} of MCh (μmol ·m^3)
Saline		14	126 ± 17	73 ± 14	3.9 ± 0.5
SR-140333	1	7	125 ± 18^a	32 ± 31^c	3.3 ± 0.3^c
Salbutamol	1	7	140 ± 31^a	51 ± 32^b	3.3 ± 0.3^c
Aminophylline	10	7	145 ± 20^a	47 ± 26^c	3.2 ± 0.3^c
Dexamethasone	0.5	7	125 ± 17^a	115 ± 45^a	3.7 ± 0.4^a
Atropine	1	7	108 ± 19^a	21 ± 30^c	3.2 ± 0.4^c

For inhibiting AR to MC aerosol (1 mmol/m^3), SR-140333 was more potent than atropine and salbutamol (Fig 1).

The ID_{50} of SR-140333 was 22 % of that of atropine, and 0.07 % of that of salbutamol (Tab 2).

Tab 2. ID_{50} and 95 % confidence limits (CL) of drugs for inhibiting response to MC aerosol. $^bP < 0.05$, $^cP < 0.01$ *vs* atropine; $^fP < 0.01$ *vs* salbutamol.

Drugs	Rats	ID_{50}/μg·kg^{-1} (nmol·kg^{-1})	95 % CL/μg·kg^{-1} (nmol·kg^{-1})
Atropine	35	22.1 (32.7)	14.4 – 34.1 (21.3 – 50.4)
SR-140333	30	4.9^{bf}(7.5)	1.4 – 17.2 (2.1 – 26.2)
Salbutamol	29	7 360^c(25 520)	3 370 – 16 040 (11 685 – 55 617)

Atropine 1 nmol · L^{-1} inhibited the contractile response of isolated tracheal strips induced by MC, but had no effect on the maximal contraction. While SR-140333 1 μmol·L^{-1} did not show significant inhibitory effect (Fig 2, Tab 3).

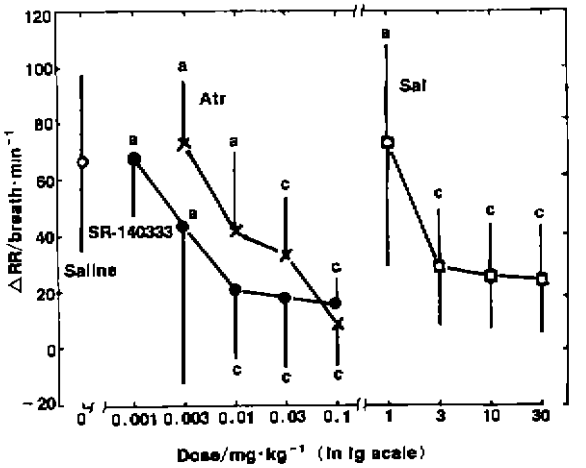

Fig 1. Effects of SR-140333, atropine, and salbutamol on quickening of breathing induced by MC aerosol (1 mmol/m³). $n = 5 - 12$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

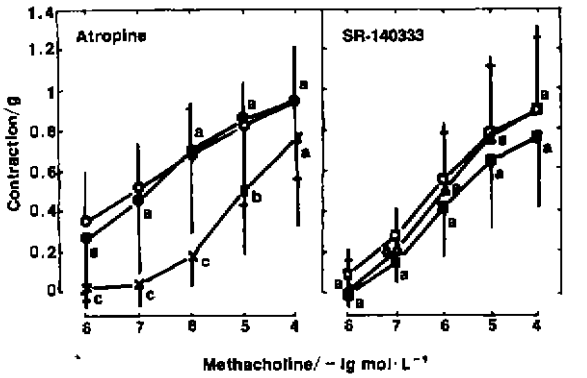

Fig 2. Effects of SR-140333 and atropine on MC-induced contraction of rat tracheal smooth muscles. Atropine at 0 (○), 0.1 (●) or 1.0 (×) nmol·L⁻¹; SR-140333 at 0 (□), 100 (■) or 1000 (△) nmol·L⁻¹; $n = 8$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

Tab 3. Effects of atropine and SR-140333 on MC-induced contraction of rat trachea. $n = 8$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* relative control.

Drugs/nmol·L⁻¹		pD_2	E_{max}/g
Control 1		7.3±0.7	0.9±0.3
Atropine	0.1	7.0±0.8[a]	1.0±0.4[a]
	1.0	5.1±0.8[c]	0.8±0.4[a]
Control 2		6.9±0.5	1.1±0.4
SR-140333	100	6.6±0.3[a]	1.0±0.4[a]
	1 000	6.6±0.4[a]	1.1±0.4[a]

DISCUSSION

In the present study, we used a non-invasive method for measuring AR in diazepam-sedated SD rats, which had advantages of less suppression on spontaneous respiration and more sensitivity to pharmacological agents (unpublished data). The increase in RR in response to inhalation of MC aerosol might result from bronchoconstriction because bronchodilators, both salbutamol and aminophylline, showed inhibitory effect, while steroid anti-inflammatory agent dexamethasone had no effect.

AR to inhaled MC *in vivo* seems to be a function of the effect of MC on airway smooth muscles and the effect is mediated by MC-induced neurohumoral regulatory pathways. Muscarinic cholinoceptor antagonist atropine blocked the AR to MC both *in vivo* and *in vitro*, indicating that the primary effect was on smooth muscles. In contrast, NK-1 receptor antagonist SR-140333 only inhibited the response to MC *in vivo*, suggesting that inhaled MC might stimulate sensory nerve endings lying adjacent to the airway lumens to release TK[3,7,8], and TK then acted on NK receptors. In the airways, NK-2 receptor activation causes direct contraction of smooth muscles, while NK-1 receptors contribute to an indirect contraction via promoting the release of inflammatory mediators, such as serotonin and histamine, from mast cells[13,14], or acetylcholine from postganglion cholinergic nerve endings[15]. These facts may explain the ability of SR-140333 to attenuate AR.

Another NK-1 receptor antagonist, CP-96345, has been found to inhibit antigen-induced bronchoconstriction and airway inflammation in sensitized guinea pigs[4-6]. Since CP-96345 also has nonspecific effect of calcium channel blockade[10], a more selective NK-1 receptor antagonist, SR-140333[9,10] has been used in this study. The results further demonstrated the inhibitory effect on AR of NK-1 receptor antagonist(s).

In conclusion, endogenous TK modulated the AR to MC *in vivo* possibly via activation of NK-1 receptors, therefore, NK-1 receptor antagonists, especially the non-peptide compounds, may be useful to inhibit AR in asthmatic patients.

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神经激肽-1 受体拮抗剂 SR-140333 对镇静大鼠乙酰甲胆碱气道反应性的作用[1]

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关键词 SR-140333; 氯醋甲胆碱; 阿托品; 神经激肽-1 受体; 沙丁胺醇; 氨茶碱; 地塞米松; 气管

目的: 探讨神经激肽对乙酰甲胆碱(MC)气道反应性的作用. **方法**: 观察非肽类 NK-1 受体拮抗剂 SR-140333 对镇静大鼠的 MC 气道反应性和离体气管条的收缩反应. **结果**: SR-140333 抑制 MC 气雾(10 - 1000 $\mu mol/m^3$)引起的呼吸频率增快, 抑制 MC 气雾(1 $mmol/m^3$)反应的 ID_{50}为 4.9 (1.4 - 17.2 $\mu g \cdot kg^{-1}$); SR-140333 1 $\mu mol \cdot L^{-1}$对乙酰甲胆碱引起的气管平滑肌收缩无抑制作用. 阿托品可阻断 MC 的在体和离体反应. **结论**: 内源性速激肽参与在体 MC 气道反应, 至少部分由 NK-1 受体介导.